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(54) Title: IGNITER FOR INTERNAL COMBUSTION ENGINES OPERATING OVER A WIDE RANGE OF AIR FUEL RATIOS

(57) Abstract: An igniter for ignition over a wide air/fuel ratio range. Igniter includes an igniter body including an internal cavity disposed substantially within the igniter body, an internal spark gap disposed substantially within the internal cavity, an external spark gap disposed substantially on an exposed surface of the igniter body, and a fuel charge delivery system for delivering a fuel charge to the internal cavity. A method for compression-igniting an air/fuel mixture in a cylinder of a internal combustion enigne, the method comprising introducing a substantially homogenous charge of a first air/fuel mixture into a cylinder of the internal combustion engine during an intake stroke, compressing the substantially homogenous charge of the first air/fuel mixture in the cylinder of the internal combustion engine during a compressin stoke, and combusting the substantially homogenous charge of the first air/fuel mixture in the cylinder of the internal combustion engine during a power stroke by injecting partially combusted products of a second air/fuel mixture into the cylinder, with the first air/fuel mixture having a substantially higher ratio, by weight, of air to fuel and the second air/fuel mixture.

WO 02/20982 A2

IGNITER FOR INTERNAL COMBUSTION ENGINES OPERATING OVER A WIDE RANGE OF AIR FUEL RATIOS

This application is being filed as a PCT International Patent Application in the name of Savage Enterprises, Inc., a U.S. national corporation and resident, (Applicant for all countries except US) and Harold E. Durling, a U.S. resident and citizen (Applicant for US only), on 07 September 2001, designating all countries and claiming priority to U.S. Serial No. 60/230,982 filed 07 September 2000.

TECHNICAL FIELD

The present invention relates generally to an igniter for use in internal combustion engines. More particularly, the invention relates to an internal combustion igniter, which permits the engine to be operated in a "spark-ignited" mode of operation (with a relatively rich fuel to air ratio) during periods of relatively heavy load and in a diesel mode of operation (with a relatively lean fuel to air ratio) during periods of relatively light load.

BACKGROUND

Internal combustion engines (i.e., those having an intake stroke, a compression stroke, a power stroke, and an exhaust stroke, either as separate strokes (four-stroke) or combined (two-stroke) events) may be divided into two general types: spark-ignited and compression-ignited (e.g., diesel).

Spark-ignited engines and compression-ignited engines each have distinct advantages and disadvantages. For example, as versus compression-ignited engines, spark-ignited engines are generally less expensive to produce, have a greater power density (i.e., horsepower produced per volume of cylinder displacement), and are usually supplied with stoichiometric air/fuel ratios that produce relatively low levels of pollutant emissions. The pollutants that are produced by spark-ignited engines run with stoichiometric air/fuel ratios can also be further reduced to currently acceptable levels by utilizing the post-combustion catalytic converter technology available today.

predicted binding affinities for MHC Class I and II have been identified using several algorithms. Predictions of Class I binding peptides were confirmed using T2 stabilization assays. The immunogenicity of the Vh peptides was tested in vitro using peptide pulsed syngeneic bone marrow-derived DCs to stimulate splenocytes. Immunogenic peptides were defined by their ability to elicit CD8 or CD4 T cell responses assessed by IFN-gamma secretion in ELISPOT assay. Eight Class I immunogenic peptides were identified, four from framework (Fr) regions. Five Class II immunogenic peptides were identified, three from Fr regions. The immunogenic Fr region peptides are from conserved germline sequences. The demonstration that conserved germline sequences in Fr regions of the Ig Vh chain are immunogenic, provides the ability to test whether such epitopes can be used to develop a more universal DC vaccine for the treatment of B-cell lymphomas in patients who share HLA alleles and lymphomas that produce Ig of the same Vh family.

162.13

NKT Cell-dependent Adjuvant Effect of Alpha-Galactosyl Ceramide in Tumor Rejection

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It has been proposed that CD1d-dependent NKT cells play an important role in innate immune response as a regulatory T cells. The role of NKT cells in establishment phase of adaptive immune response was investigated with a specific glycolipid ligand, alpha-galactosyl ceramide, which is presented to NKT cells on CD1d in animal tumor rejection model.

Alpha-galactosyl ceramide showed a strong adjuvant effect against male specific minor antigen H-Y and tumor specific antigens. Adjuvant effect was totally abolished in CD1d-/- mice where NKT cell development is impaired. NKT cell-dependent protective immunization against live tumor cells also required MHC class II-dependent CD4+ T cells and NK cells implying that activated NKT cells exert its effect by promoting adaptive immune response where CD4+ T cells are major effecter.

Collectively, our data demonstrate that activation of NKT cells at the step of immunization can greatly improve vaccination effect against inefficient antigens.

162.14

A recombinant vector expressing transgenes for four T-cell costimulatory molecules (OX40L, B7-1, ICAM-1, LFA-3) induces sustained CD4+ and CD8+ T-cell activation, protection from apoptosis and enhanced cytokine production

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The role of OX40L on the activation of T cells was investigated using poxvirus vectors expressing OX40L alone or in combination with three other T-cell costimulatory molecules: B7-1, ICAM-1, and LFA-3. Poxvirus vector-infected cells were used to stimulate naïve or activated CD4+ and CD8+ T cells. The effect of poxvirus-vectored costimulatory molecules on the activation of T cells was determined by proliferation and cytokine production. Additionally, apoptosis levels, as well as expression of genes involved in apoptosis (both pro-apoptotic and inhibitory) were analyzed following T-cell activation. These studies demonstrate that a) OX40L plays a role in sustaining the long-term proliferation of CD8+ T cells in addition to the known effect on CD4+ T cells following activation, b) OX40L enhances the production of cytokines (IL-2, IFN-γ and TNF-α) from both CD4+ and CD8+ wh change in IL-4 expression was observed, and c) the anti-apoptotic of OX40L on T cells is likely the result of elevated expression of apoptotic genes while genes involved in apoptosis are inhibite addition, these are the first studies to demonstrate that the combine of a vector driving the expression of OX40L with three costimulatory molecules (B7-1, ICAM-1 and LFA-3) both enh initial activation and then further potentiates sustained activati naïve and effector T cells.

162.15

Dendritic Cell Immunization Route Determines the Location of T cell Activation, Patterns of Memory T cell Homing, and Anti-tumor Efficacy

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We established that the route by which peptide antigen-pulsed, activated DC are introduced leads to differences in the distribution of primary and memory T cell immunity, and affects the ability to control the outgrowth of tumors in different sites in the body. Subcutaneously-injected DC migrated in small numbers to draining peripheral lymph nodes (LN), but were largely found in spleen. In contrast, intravenously-injected DC were found only in spleen and not in any peripheral or mucosal LN compartments. Primary $CD8^+$ T cell responses measured 7 days later were confined to those compartments into which the DC had infiltrated. Memory CD8 cells induced in spleen by IV immunization continued to be confined to that organ and absent from peripheral and mucosal LN. Conversely, subcutaneous immunization with DC led to memory $CD8^+$ T cells located in peripheral and mucosal LN as well as spleen. Several lines of evidence suggest that T cells in peripheral LN were necessary for control of melanoma growing subcutaneously while T cells in spleen were sufficient to control tumors growing in the lung. Collectively, these data suggest that regional immunization may give rise to distinct populations of LN- and spleen-homing memory T cells. These studies provide a basis for improvements in tumor immunotherapy and an understanding of T cell homing and regional immunity in general.

162.16

Induction of anti-GD2 ganglioside antibody responses by a GD2 ganglioside peptide mimic

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Previous studies have suggested that the induction of autologous anti-GD2 Abs in patients with neuroblastoma, following administration of mouse anti-GD2 mAb 3F8, was associated with patients' long-term survival. This has been suggested to reflect the triggering of the idiotypic (id) cascade and is paralleled by the clinical observation that anti-id mAb can induce anti-GD2 Abs in patients with neuroblastoma. The immunogenicity of anti-id mAb, appears to be higher than that of KLH-conjugated GD2, suggesting that mimics of GD2 may represent useful immunogens to implement active specific immunotherapy of melanoma and neuroblastoma. To circumvent the adverse side effects associated with administration of anti-GD2 mAbs, the goal of this work is to induce a anti-GD2 immune response using GD2 peptide mimics, since peptide mimics have advantages in terms of their production, standardization, modification and antigen presentation. In this study, we have isolated a GD2 peptide mimic, J51, which inhibits the binding of mAb 3F8 to GD2(+) cells. The immunogenicity of J51 was tested by immunizing BALB/c mice with KLH-conjugated cyclic peptide J51 and Freund's adjuvant. After the 5th immunization, IgG Abs, which specifically react with GD2(+) cells were detected in all immunized mice. These results suggest that peptides can mimic GD2 and induce anti-GD2 IgG Ab responses in BALB/c mice. This work was supported by PHS grant RO1 CA37959, awarded by the National Cancer Institute, DHHS.

162.17

Recombinant L. monocytogenes as a vaccine for stimulation of anti-tumor responses.

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We have compared the stimulation of anti-tumor responses following injection with attenuated strains of Lm expressing a model tumor

antigen (SIINFEKL) in the C57BL/6 B16 melanoma model system. Our results suggest that virulence attenuation (e.g., actA-) does not compromise the prophylactic or therapeutic effectiveness of Lm as a cancer vaccine. Animals immunized with recombinant Lm and then challenged with SIINFEKL expressing melanoma cells do not show an expansion of SIINFEKL-specific T-cells in the spleen, as might be expected for a secondary response. We also do not observe proliferation of antigen-specific cells following co-culture with melanoma cells in vitro. We have derived tumor antigen-specific effector cells from immunized mice and have stimulated these cells under in vitro culture conditions that allow for the selection of T-cell populations that can respond to differing levels of the peptide target (1 nM vs 100 nM). These in vitro selected T-cell subsets have differing capacities to recognize peptide pulsed targets and tumor targets by ELISPOT assays, and appear to possess differing anti-tumor effector function following adoptive transfer into tumor bearing hosts. We are using the selected high affinity effector populations to assess their anti-tumor efficacy against isolated variants that phenotypically express reduced levels of MHC Class I. Supported by VA Merit Review, NIH and Cerus Corp.

162.18

Comparison of Antigen-Specific T cell Responses Induced by Transduction of Human Dendritic Cells with E1- and E1-, E2b- Adenoviral Vectors: Development of Adenovirus Vectors for DC-Based Anti-Tumor Immunotherapy

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Antigen loading of dendritic cells (DC) by gene modification is a promising method for eliciting antigen-specific immune responses *in vivo*. Adenoviral (Ad)-mediated gene transfer is an efficient DC transduction method; however, limitations exist to Ad-mediated vaccine approaches using conventional E1-deleted vectors, including vector replication and toxicity due to Ad late gene expression. We have developed an Ad vector deleted for both the E1 and E2b gene functions, resulting in a non-replicative vector from which Ad late gene expression is significantly impaired (Amalfitano A, et al. J Virol. 72:2, 1998). To test the impact [E1-, E2b-] Ad vectors have on elicitation of immune responses, we produced [E1-] and [E1-, E2b-] Ad vectors that express the CMV pp65 lower matrix protein. Flow cytometric analysis reveals that both [E1-] and [E1-, E2b-] Ad vectors consistently transduce human DC to high levels, as well as induce DC maturation. Moreover, DC transduced with either vector are capable of generating robust anti-CMVpp65 T-cell responses *in vitro*. These findings indicate that [E1-, E2b-] Ad vectors have potential for successful therapeutic use, having similar antigen expression, but reduced toxicity, compared with conventional Ad vectors. Similar studies will test [E1-, E2b-] Ad vectors expressing CEA, HER-2/neu, or WT-1 tumor associated antigens for future use in anti-tumor immunotherapy trials.
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162.19

Mouse CD20 as a model target for immunotherapy requires Fc receptor-dependent cell-mediated effector functions that are independent of complement-mediated cytotoxicity

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CD20 is a B cell-specific surface molecule with four membrane-spanning regions. While CD20 is a well characterized antigen and target for immunotherapy in humans, relatively little is known about the CD20 molecule in mice. We have therefore examined CD20 expression and function using a panel of anti-mouse CD20 monoclonal antibodies. CD20 was first expressed by some late-stage pre-B cells and most of the immature B cells in bone marrow, but was expressed by the majority of mature B cells in the periphery. Mouse CD20 was expressed as a 33 and 35 kDa protein that was phosphorylated following B cell activation. In vivo, anti-CD20 monoclonal antibodies depleted greater than 93% of peripheral cells in wild type mice through an antibody isotype-dependent mechanism. B cell clearance predominantly required mouse expression of the Fc receptor common gamma chain, but did not depend on C3 complement expression. These results demonstrate that human and mouse CD20 have similar expression patterns. Moreover, these studies reveal that anti-CD20 antibody-based immunotherapy for lymphoma and autoimmunity is likely to depend on antibody-dependent cell-mediated effector functions that are independent of complement-mediated cytotoxicity.

162.20

Type-1 polarized DC Obtained in Serum-Free Conditions are Powerful Inducers of Anti-melanoma Responses

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Type-1 polarized DCs (DC1s) show a unique combination of a fully-mature status and an elevated, rather than "exhausted", ability to produce IL-12. This results in their selectively enhanced ability to induce type-1 immunity, desirable in cancer. Clinical application of DC1s has been hampered by the lack of clinically-acceptable protocols of DC1 generation in FCS-free culture conditions. We have recently developed three novel maturation-inducing cytokine cocktails allowing us to generate fully-mature DC1s in serum free AIM-V medium. A single round of *in vitro* sensitization with serum-free DC1s loaded with MART-1-, gp100- and tyrosinase-derived peptides, followed by expansion of the resulting cell lines with autologous PBMC, allows for the induction of 5-60 fold higher numbers of $CD8^+$ T cells specific for the individual peptides (IFNγ ELISPOT), compared with the parallel cultures employing TNFα/IL-1β/IL-6/PGE_2-matured DCs, the current "gold standard" of DC-based cancer vaccines. In case of MART-1, the frequencies of the DC1-induced peptide-specific T cells reached 2-10% of total $CD8^+$ T cells, being inducible both in melanoma patients and in healthy donors. High CTL-inducing activity of DC1s requires the presence of CD40L-mediated helper signals from $CD4^+$ T cells. The availability of serum-free protocols of DC1 generation allows for clinical application of DC1-based vaccines in melanoma and other tumors. *Supported by grants from NIH (CA82016) and Pittsburgh Foundation (to PK).*

162.21

NFkB inhibition in tumor cells contributes to Dendritic Cell activation and subsequent Peripheral Blood Lymphocyte Activation

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Objective: To study the role of NFkB nuclear binding activity in tumor cell mediation of immune effector cell inactivation and depletion.
Methods: DC's were left untreated or treated with a combination of IFN-g and LPS before their exposure to vector alone and IkB-super repressor transfected Hep-2 cells (HEp2-IkB(S32AS36A)). Supernatants for co-culture of DC and Hep-2 transfectants were removed and assayed for TNF-a and IL-12 secretion. DCs were then co-cultured in the presence of untreated and IL-2 treated PBLs and their functional activation was determined by measuring secretion of TNF-αAND GMCSF.
Results: Increased IL-12 and TNF-a secretion was observed when a combination of LPS and IFN-g treated DCs were co-cultured in the presence of IkB-super repressor transfected Hep-2 cells (HEp2-IkB(S32AS36A)) as compared to vector alone transfected Hep-2 cells. DC+PBL with HEp2-IkB(S32AS36A) tumor cell co-cultures show greater PBL activation than those cultures co-incubated without DC's or with vector-alone transfected tumor cells.
Conclusions: LPS-treated DC's exposed to tumor cells lacking NFkB (HEp2-IkB(S32AS36A)) show greater activation than controls. Accordingly, DC's contribute to the activation of PBLs significantly when tumor cells are devoid of NFkB activity.